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EXAMINER	
NGUYEN, QUANG	
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19

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Applicati n No. 09/487,318	Applicant(s) REID ET AL.	
	Examiner Quang Nguyen, Ph.D.	Art Unit 1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 September 2002* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-6,8,9,11-35,38,39 and 42-48* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-6, 8-9, 11-35, 38-39 and 42-48* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Applicants' amendment filed on 9/30/02 in Paper No. 16 has been entered.

Amended claims 1-6, 8-9, 11-35, 38-39, 42-46 and new claims 47-48 are pending in the present application.

The text of those sections of Title 35 U.S.C. Code not included in this action can be found in a prior Office Action.

Claim Rejections - 35 USC § 112

Amended claims 27-35 and 39 remain rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention, for the same reasons set forth in the previous Office Action.

Amended claims 27-34 are directed to a method of treating liver dysfunction or disease responsive to treatment with liver progenitors, comprising administering to a subject in need of such treatment an effective amount of human liver progenitors, their progeny, more mature forms thereof, or combinations thereof, in a pharmaceutically acceptable carrier and treating the liver dysfunction or disease.

Amended claim 35 is directed to a method of treating a disease comprising administering to a subject in need of such treatment an effective amount of human hepatic progenitors, their progeny, or more mature forms thereof in which the human

hepatic progenitors, their progeny, or more mature forms harbor exogenous nucleic acid.

Amended claim 39 is drawn to a pharmaceutical composition comprising an enriched population of human hepatic pluritotential progenitors, their progeny, or more mature forms thereof, which exhibit one or more markers indicative of expression of full-length alpha-fetoprotein, albumin, or both; and a pharmaceutically acceptable carrier.

The specification teaches by exemplification the isolation of human liver progenitor cells from fetal and adult human livers. With regard to the nature of the instant claims, example 10 of the specification discloses that hepatic damage in C57BL/6 mice was induced by perfusion of hepatic progenitors infected with recombinant adenovirus expressing human urokinase plasminogen activator (uPA) via the portal vein. By day 3, treated mice had a moderate inflammatory infiltrate comprising macrophages and neutrophils, and degenerative changes in hepatocytes were observed. Eight to 10 days after the transplantation of the infected hepatic progenitors, it was reported that there was evidence of hepatic recovery (e.g., presence of multifocal regeneration, heterologous size of nucleic, decreased inflammatory reactions with few degenerative hepatocytes). It was suggested that the urokinase expression in combination with hepatic progenitors induced significant liver parenchymal cell regeneration. The above evidence has been noted and considered. However, it is not reasonably extrapolated to the instant claimed invention.

The specification is not enabled for the instant claimed invention because it fails to provide sufficient teachings and guidance demonstrating that by administering human

liver progenitors of the present application into a host or a subject having any liver dysfunction or disease, the subject would be treated for symptoms associated with the liver dysfunction or disease. At the effective filing date of the present application, the art on transplantation of liver progenitors for treating liver dysfunction or disease was still immature and highly unpredictable, particularly for attaining the desired therapeutic effects (Shafritz, Hepatology, pages 1399-1400, 2000; IDS; Vessey et al., Pathology 33:130-141, 2001; see the section entitled "Future applications of hepatic stem cells"). Shafritz state that "...liver transplantation is the only available current therapy for end-stage liver failure.....finding alternative methods for liver replacement is of utmost importance. One such method would be functional repopulation of the diseased liver by cell transplantation." (page 1399, column 1, lines 1-6). Thus, it is apparent that a method for treating any liver dysfunction in a subject using cell transplantation, including human liver progenitors of the instant invention, is not achievable or routine or predictable even in the year 2000, let alone at the effective filing date of the present application (January 19, 1999). Additionally, Shafritz et al. further note that "the clonality and bipotent nature of isolated liver stem/progentior cells need to be confirmed by *in vivo* transplantation studies" because there is no consistent data in the art (page 1400, col. 1, middle paragraph, lines 12-26). Furthermore, there is no apparent correlation between an increase in the uptake of hepatic 3H-thymidine in mice treated with hepatic progenitors infected with recombinant adenovirus expressing human urokinase plasminogen activator (uPA) to obtaining any therapeutic effects contemplated by the treatment methods claimed. There is also no evidence of record

indicating that the transplanted hepatic progenitors give rise directly to regeneration of parenchymal cells, and not due to the proliferation of endogenous hepatocytes. Thus, there is a lack of a nexus between a specific given example provided by the specification and the methods as claimed. In addition, the specification fails to provide specific relevant information such as the effective cell dosages, the frequency of administration and the exact site of introduction for a given specific liver dysfunction or disease to obtain any therapeutic effects contemplated by Applicants.

The instant claims encompass autologous, allogeneic as well as xenogenic transplantation of human liver progenitors into a subject having liver dysfunction or disease. There is no evidence indicating that that the delivered human liver progenitors, their progeny or mature forms thereof would be free from the adverse host immune reactions. It is already well known in the art that adverse host immune rejection reactions present a formidable challenge in the transplantation of allogeneic and particularly xenogeneic cells and tissues. There is no evidence of record indicating that the newly introduced human liver progenitors or hepatic pluripotent progenitors would be properly engrafted, proliferated, and differentiated into mature and functional liver cells in an effective amount or any mature functional cells derived from human liver progenitors to yield the desired therapeutic effects in any treated subject. Even in the exemplified example, moderate inflammatory responses were noted and persisted for 3 to 4 weeks in the mouse model, and there was no evidence indicating that the administered liver progenitors proliferate, differentiate into functional liver cells.

The instant claims also encompass any administering routes for the delivery of liver progenitors into a subject having a liver dysfunction or disease. However, it is unclear whether effective levels of liver progenitors can home in to a diseased or disordered liver to proliferate and differentiate into functional mature cells (liver cells or any mature cells derived from human liver progenitors) to yield the desired therapeutic effects. Moreover, Dabeva et al. (Am. J. Pathology 156:2017-2031, 2000; IDS) have stated "Several studies also report successful engraftment and differentiation of early fetal liver tissue or cell suspensions after transplantation into ectopic sites. However, engrafted liver tissue masses at ectopic sites do not expand very much, and it is unlikely that such limited liver transplantation will have broad therapeutic application" (page 2029, column 2, last 3 sentences continue to lines 1-4 of column 1, page 2030). The mere mentioning of advantages offered by human liver progenitors for *ex vivo* gene therapy and routes of tranplantation (See specification, pages 40-44) is not seen as providing enablement as there is no correlation between these and any therapeutic outcome. Without the specific teaching or guidance provided by the specification, it would have required undue experimentation for one skilled in the art to make and use the instant claimed invention, particularly in light of the state of the art of human liver progenitor cell transplantation therapy. The CAFC has stated "patent protection is granted in return for an enabling disclosure, not for vague intimations of general ideas that may or may be workable". The court continues to state that "tossing out the mere germ of an idea does not constitute an enabling disclosure" and that "the specification, not knowledge in the art, that must supply the novel aspects of an invention in order to

constitute adequate enablement". (See *Genetech, Inc. v. Novo Nordisk A/S*, 42 USPQ 2d 1001, at 1005).

With regard to the breadth of claim 35 encompassing human hepatic progenitors, their progeny, or more mature forms thereof comprising any exogenous nucleic acid to treat any disease in a subject, the specification fails to teach any specific vector used to deliver and express a specific gene (a therapeutic protein) in human hepatic progenitor cell populations of the instant claimed invention for treating a specific disease. In addition to the obstacles of a human liver progenitor cell transplantation therapy discussed in the preceding paragraphs, at the effective filing date of the present application, it has been noted that sub-optimal vectors, the lack of long-term and stable transgene expression are some of the factors limiting an effective gene therapy. In a review on gene delivery systems available for gene therapy (Methods of gene delivery, Hematol. Oncol. Clin. North Am. 12:483-501, 1998), Wivel and Wilson stated that "One of the major challenges still confronting the field is the design of more efficient vectors. The gene delivery systems being used today will undoubtedly be seen as crude when compared with future developments. It is unlikely that there will ever be a universal vector, but rather there will be multiple vectors specifically designed for certain organ sites and certain diseases... It will be necessary to do much more fundamental research in cell biology, virology, immunology, and pathophysiology before vectors can be significantly improved." (pages 498-499 in Summary section). On the basis of the instant disclosure, there is no evidence of record that human hepatic progenitors, their progeny or more mature forms thereof haboring exogenous nucleic acid could persist in

a treated subject for a sufficient period of time to provide a stable, long-term *in vivo* expression of therapeutic transgenes to yield the therapeutic outcomes for any diseases. As written, the claims do not even require any expression of any therapeutic genes since the cells merely harbor exogenous nucleic acid that is not necessarily coupled to any promoter or enhancer. *In vivo* expression of therapeutic transgenes is known to be transient. This is supported by numerous teachings in the art. As examples, Palmer et al. (Proc. Natl. Acad. Sci. 88:1330-1334, 1991) demonstrated that the *in vivo* expression of human factor IX by transplanted syngeneic recombinant fibroblasts was transient and vanished 1-5 weeks post-transplantation. Riddell et al. (Nature Med. 2:216-223, 1996) reported that five out of six patients seropositive for human immunodeficiency virus quickly developed cytotoxic T-lymphocytes responses specific to a novel protein and eliminated infused autologous CD8+ HIV-specific cytotoxic T cells transduced with a fusion suicide gene (See abstract). Additionally, factors such as the level of mRNA produced, the stability of the protein produced, the protein's compartmentalization within the cell or its secretory fate differ dramatically based on which protein being produced, and therefore the desirable therapeutic effect sought to achieve. Thus, the level of gene expression, its duration, and its *in vivo* therapeutic effects are not always predictable, and they can not be overcome with routine experimentation. Accordingly, with the lack of guidance provided by the instant specification, it would have required undue experimentation for one skilled in the art to make and use the method as claimed.

With respect to claim 39 directed to a pharmaceutical composition comprising an enriched population of human hepatic pluripotent progenitors, their progeny or more mature forms thereof, the instant specification is not enabled for the use of said composition to treat liver dysfunction or disease for the same reasons discussed above. It is noted that enablement requires the specification to teach how to make and use the claimed invention.

Accordingly, due to the lack of direction or guidance presented in the specification regarding to the issues set forth above, the unpredictability of the human liver progenitor cell transplantation therapy and gene therapy arts, the absence of working examples, and the breadth of the claims, it would have required undue experimentation for one skilled in the art to make/use the claimed invention.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on 9/30/02 in Paper No. 16 (pages 11-15) have been fully considered.

Applicants argue that the instant specification provides sufficient guidance for the use of the isolated progenitors of the invention and their progeny for the treatment of liver diseases and dysfunctions as claimed by referring to various passages in the application. Applicants maintain that the administration of suspensions of hepatocytes to subjects is routine, and that Applicants discussed the advantages hepatic progenitors versus hepatocytes with regard to cell size, immunological rejection and proliferative capacity for treatment purposes. Applicants further argue that Examiner imposes a

utility requirement that the claimed method of treatment be fully effective and safe for patentability purposes. Examiner respectfully finds Applicants' arguments to be unpersuasive for the following reasons.

Firstly, the mere general description or mentioning the advantages offered by human liver progenitors for treating a host of liver dysfunction, disorders and diseases such as hepatocholangitis, cirrhosis, hepatitis, acute and chronic liver failure, hepatocarcinoma, hepatoblastoma and others, is not seen as providing enablement as there is no correlation between these general descriptions and contemplated therapeutic outcomes. Without specific guidance provided by the specification, it would have required undue experimentation for one skilled in the art to make and use the methods as claimed. It should be noted that the physiological art is recognized as unpredictable (MPEP 2164.03), especially for achieving therapeutic effects for a whole host of liver diseases and disorders as claimed. In contrary to Applicants' simple assertion that once the hepatic progenitors are administered into a subject suffering from a liver dysfunction or disease, they then go about the business of alleviating the medical condition affecting the liver, there are several issues that the specification fails to address such that contemplated therapeutic outcomes could be attained without undue experimentation. As noted in the previous Office Action, there is no evidence indicating that the introduced human liver progenitors are properly engrafted, proliferated, and differentiated into mature and functional liver cells in an effective amount in any treated subject to yield any therapeutic outcomes or effects. As recited, the claims encompass syngeneic, allogeneic and xenogenic transplantation of human

liver progenitors into a subject in need of treatment. There is no evidence indicating that that the delivered human liver progenitors, their progeny or mature forms thereof would be free from the adverse host immune reactions. It is already well known in the art that adverse host immune rejection reactions present a formidable challenge in the transplantation of allogeneic and particularly xenogeneic cells and tissues. Even in the exemplified example, moderate inflammatory responses were noted and persisted for 3 to 4 weeks in the mouse model, and there is no evidence indicating that the administered liver progenitors proliferate, differentiate into functional liver cells. The instant claims also encompass any administering sites for the delivery of liver progenitors into a subject having a liver dysfunction or disease. However, it is unclear whether effective levels of liver progenitors can home in to a diseased or disordered liver to proliferate and differentiate into functional mature cells to yield therapeutic effects by any and all routes of administration. Moreover, Dabeva et al. (Am. J. Pathology 156:2017-2031, 2000; IDS) have stated "Several studies also report successful engraftment and differentiation of early fetal liver tissue or cell suspensions after transplantation into ectopic sites. However, engrafted liver tissue masses at ectopic sites do not expand very much, and it is unlikely that such limited liver transplantation will have broad therapeutic application" (page 2029, column 2, last 3 sentences continue to lines 1-4 of column 1, page 2030). In a more recent review on liver stem cell, Shafritz (Hepatology, pages 1399-1400, 2000; IDS) state "...liver transplantation is the only available current therapy for end-stage liver failure.....finding alternative methods for liver replacement is of utmost importance.

One such method would be functional repopulation of the diseased liver by cell transplantation." (page 1399, column 1, lines 1-6). So clearly, a method for treating any liver dysfunction in a mammal using cell transplantation, including human liver progenitors of the instant invention, is not achievable or routine or predictable even in the year 2000, let alone at the effective filing date of the present application (January 19, 1999). Interestingly, Shafritz et al. further note "the clonality and bipotent nature of isolated liver stem/progentior cells need to be confirmed by *in vivo* transplantation studies" because there is no consistent data in the art (page 1400, col. 1, middle paragraph, lines 12-26). Accordingly, given the lack of guidance provided by the instant specification, particularly in the absence of any relevant *in vivo* example, coupled with the unpredictability of the physiological art, the state of liver progenitor cell transplantation, and the breadth of the instant claims, it would therefore have required undue experimentation for a skilled artisan to make and use the methods as claimed.

Secondly, with respect to Applicants' argument that the Examiner imposes a utility requirement that the claimed method of treatment be fully effective and safe for patentability purposes, this is not true. The above rejection is an enablement rejection and not a utility rejection, and that the instant specification fails to enable a skilled artisan to obtain any therapeutic effects in treating a host of liver dysfunction, disorders contemplated by Applicants for the methods as claimed for the reasons discussed above which are based on the Wands factors.

With regard to claim 35, Applicants basically argue that the specification provides sufficient guidance regarding to providing hepatic precursors with an exogenous gene of

interest for treatment purposes by citing various passages in the specification and example 10. Additionally, Applicants argue that the Examiner focuses on how the available vector in the art might not fully effective to enable the instant claimed invention. Applicants' arguments are found to be unpersuasive. This is again because the general description in the specification is not deemed to be equivalent to the therapeutic outcomes contemplated by the method as claimed. Furthermore, the specification is not enabled for the claimed method for the reasons stated in the preceding paragraphs, and not on the lack of optimal vectors available for gene therapy. Additionally, there are issues such as whether the genetically modified human hepatic progenitors, their progeny or mature forms thereof could be stably engrafted, differentiated and proliferated into functional liver cells could be achieved in the treated subject and whether said cells persist in a treated subject for a sufficient period of time to provide a stable, long-term *in vivo* expression of therapeutic transgenes to yield therapeutic outcomes for any and all diseases. The nature of the claim 35 falls within the realm of *ex vivo* gene therapy which was highly unpredictable at the effective filing date of the present application with respect to achieving therapeutic results. As noted in the previous Office Action, factors such as sub-optimal vectors, the lack of a long-term and stable *in vivo* transgene expression are some of the factors limiting the effectiveness of *ex vivo* gene therapy. For examples, Palmer et al. (Proc. Natl. Acad. Sci. 88:1330-1334, 1991) demonstrated that the *in vivo* expression of human factor IX by transplanted syngeneic recombinant fibroblasts was transient and vanished 1-5 weeks post-transplantation. Riddell et al. (Nature Med. 2:216-223, 1996) reported that

five out of six patients seropositive for human immunodeficiency virus quickly developed cytotoxic T-lymphocytes responses specific to a novel protein and eliminated infused autologous CD8+ HIV-specific cytotoxic T cells transduced with a fusion suicide gene (See abstract). Given the unpredictability of the gene therapy art coupled with the lack of specific guidance provided by the instant specification with respect to attaining therapeutic effects for any and all disease in a host by overcoming the aforementioned factors, it would have required undue experimentation without a predictable expectation of success for one skilled artisan to make and use the instant claimed invention.

Accordingly, claims 27-35 and 39 remain rejected under 35 U.S.C. 112, first paragraph for the reasons set forth above.

Following is a new ground of rejection necessitated by Applicants' amendment.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Amended claims 11-20 and 44 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In claim 11, the phrase "A human hepatic pluripotent progenitor isolated by the method of claim 1" is unclear. This is because the method of claim 1 provides a mixture

of immature cells comprised of an enriched population of human liver progenitors, and the method does not recite any essential steps required to obtain a human hepatic pluripotent progenitor from a mixture of immature cells, therefore it is unclear how a human hepatic pluripotent progenitor could be isolated or obtained from such a method. Clarification is requested.

In claim 12 and its dependent claims, it is unclear what is the nexus of obtaining a mixture of cells which is comprised of an enriched population of human liver progenitors in step (c) with the preparation of a composition comprising an enriched population of human hepatic progenitors recited in the preamble of the claim. Clarification is requested because the metes and bounds of the claims are not clearly determined.

Claim 14 and its dependent claims recite the limitation "the immunoselection" in line 1 of claim 14. There is insufficient antecedent basis for this limitation in the claim. There is no recitation of any immunoselection step in the amended claim 12 from which claim 14 is dependent upon.

Claim 44 recites the limitation "The isolated human liver progenitors" in line 1 of the claim. There is insufficient antecedent basis for this limitation in the claim. There is no recitation of human liver progenitors in the amended claim 43 from which claim 44 is dependent upon.

Claim R jections - 35 USC § 102

Amended claims 21-23 and 42-44 are rejected under 35 U.S.C. 102(b) as being anticipated by Muench et al. (Blood 83:3170-3181, 1994) or Muench et al. (Blood 89:1364-1375, 1997).

Claims 21-23 are drawn to a composition comprising an enriched population of human hepatic pluripotent progenitors, their progeny, or more mature forms thereof, which exhibit one or more markers indicative of expression of full-length alpha-fetoprotein, full-length albumin, or both; the same composition wherein the progenitors comprise hepatic progenitors, hematopoietic progenitors, mesenchymal progenitors, or combinations thereof, and the same wherein the progenitors, their progeny, or more mature forms thereof express CD14, CD34, CD38, CD117, ICAM or combinations thereof. Claim 42 is directed to isolated human hepatic pluripotent progenitors, their progeny or more mature forms whereof which exhibit one or more markers indicative of expression of alpha-fetoprotein, albumin, or both. Claims 43-44 are drawn to isolated human hepatic pluripotent progenitors, their progeny or more mature forms thereof which exhibit the phenotype glycophorin A^-, $CD45^-$, alpha-$fetoprotein^{+++}$, $albumin^+$, and $ICAM^+$, and the same which further express $CD14^+$, $CD34^{++}$, $CD38^{++}$, $CD117^{+++}$, or combinations thereof.

Muench et al. (1994) disclose the isolation of human fetal liver progenitors with a high proliferative potential and a phenotype of $CD34^+$, $CD33^+$, $CD13^+$, CD38-, lin^- (lineage= CD3, CD8, CD10, CD14, CD15, CD16, CD19, CD20 and glycophorin A), $CD45RA^-$, $CD45RO^-$, $CD71^-$, and heterogeneous *for c-kit* or CD117 (See abstract and

page 3171). Additionally, Muench et al. (1994) disclose the isolation of $CD34^+$, $CD33^-$ HLA-DR-, CD38- cell population previously suggested to contain stem cells (see abstract and page 3179, col. 2). Muench et al. (1997) disclose the isolation of hematopoietic stem cells derived from human fetal liver, with a phenotype of $CD4^+$, $CD34^{++}$, Lin^-, $CD117^+$, $CD38^-$, $CD45RA^-$ (See abstract and page 1365). As the human hepatic pluripotent progenitors of the presently claimed invention share the same common cell surface antigens, and are indistinguishable over the isolated human fetal liver progenitor cell populations of Muench et al. (1994) and Muench et al. (1997), the references anticipate the instant claims.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on 9/30/02 in Paper No. 16 (page 7) have been fully considered.

Applicants argue that while hepatic progenitors and hematopoietic progenitors may have common cell surface antigens, Muench references never teach or suggest that they had isolated hepatic progenitors, and at best they only had isolated hematopoeitic progenitors. Applicants' arguments are respectfully found to be unpersuasive because the human hepatic pluripotent progenitors of the presently claimed invention are indistinguishable over the isolated human fetal liver progenitor cell populations of Muench et al. (1994) and Muench et al. (1997) by sharing the same common cell surface antigens. It is further noted that a product and its properties can

not be separated. Moreover, the human hepatic pluripotent progenitors of the presently claimed invention comprise hemopoietic progenitors.

Accordingly, claims 21-23 and 42-44 are rejected for the reasons set forth above.

Amended claims 1-2, 4-6, 8-9, 11-14, 18-23, and 42-44 are rejected under 35 U.S.C. 102(b) as being anticipated by Craig et al. (J. Exp. Med. 17:1331-1342, 1993).

Craig et al. disclose the isolation of human hematopoietic progenitor cells derived from human fetal liver with a phenotype of Thy-1^{+}, $CD34^{+}$, $CD38^{low}$, $CD45RA^{-}$, $CD45RO^{+}$, $CD71^{low}$, and $CD117^{low}$ (See abstract, and column 1, second paragraph, page 1332). The method comprises the preparation of low density mononuclear cells by density centrifugation using Ficoll-Paque (column 1, page 1332, lines 24-26). In some samples, red blood cells were lysed by the addition of 10-fold excess of ammonium chloride lysing solution (column 1, page 1332, lines 30-32). Subpopulations of low density mononuclear cells were subsequently sorted by multiparameter flow cytometry, a form of positive immunoselection (column 2, page 1335, second paragraph). As the method of Craig et al. (1993) is indistinguishable from the method of the instant claims, the method of Craig et al. inherently produces an enriched mixture of cells comprised of an enriched population of human liver progenitors and/or human heptatic progenitors as claimed. Moreover, the human hepatic pluripotent progenitors of the presently claimed invention are indistinguishable over the isolated human fetal liver hematopoietic cell populations of Craig et al. because they share the same common cell surface antigens. Furthermore, the human liver progenitors and/or hepatic

pluripotent progenitors of the presently claimed invention comprise hemopoietic progenitors.

Thus, the reference anticipates the instant claims.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on 9/30/02 in Paper No. 16 (pages 7-8) have been fully considered.

Applicants argue basically that Craig's failure to disclose the isolation of human hepatic progenitors, and therefore Craig does not anticipate the instant claims. Applicants' argument is respectfully found to be unpersuasive because the method of Craig et al. (1993) is indistinguishable from the method of the instant claims, therefore the method of Craig et al. inherently produces an enriched mixture of cells comprised of an enriched population of human liver progenitors and/or human heptatic progenitors as claimed. Moreover, the human hepatic pluripotent progenitors of the presently claimed invention are indistinguishable over the isolated human fetal liver hematopoietic cell populations of Craig et al. because they share the same common cell surface antigens. Furthermore, the human liver progenitors and/or hepatic pluripotent progenitors of the presently claimed invention comprise hemopoietic progenitors.

Accordingly, claims 1-2, 4-6, 8-9, 11-14, 18-23 and 42-44 are rejected for the reasons set forth above.

Amended claims 11, 20, 21-26 and 42-44 rejected under 35 U.S.C. 102(e) as being anticipated by Faris (U.S. Patent No. 6,129,911 with an effective filing date of 7/10/1998).

Claims 21-26 are drawn to a composition comprising an enriched population of human hepatic pluripotent progenitors, their progeny, or more mature forms thereof, which exhibit one or more markers indicative of expression of full-length alpha-fetoprotein, full-length albumin, or both; the same composition in which progenitors harbor exogenous nucleic acid promoting the expression of at least one polypeptide of interest. Claims 11 and 20 are directed to a human hepatic pluripotent progenitor isolated by the methods of claims 1 and 14, respectively. Claims 42-43 are drawn to isolated human hepatic pluripotent progenitors.

Faris teach the preparation and isolation of a liver cell cluster of less than 10 cells comprising a liver stem cell and a hepatocyte, and a primary liver stem cell derived from human liver tissue, in which said stem cell comprises DNA encoding a heterologous polypeptide, such as ornithine transcarbamylase, glutamine synthetase, Factor XIII, Factor IX and others (See columns 1-3, and the claims). The primary liver stem cell derived from human liver tissue is defined as undifferentiated cell that differentiates into a mature functional hepatocyte or bile duct cell (column 1, lines 37-39) which is consistent with the definition of hepatic progenitors of the instant claimed invention (cells give rise to hepatocytes and biliary cells, page 22, lines 3-4). Since a product and its properties can not be separated, the composition of isolated liver cell cluster of Faris is the same as enriched population of human hepatic pluripotent progenitors, their

progeny or more mature forms thereof, or isolated human hepatic pluripotent progenitors of the instant invention, regardless how they are isolated, the reference therefore anticipates the instant claims.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on 9/30/02 in Paper No. 16 (pages 8-9) have been fully considered.

Applicants argue that Farris may have demonstrated proliferative characteristics, he has not demonstrated neither pluripotent nor hepatic characteristics. The failure to confirm that the isolated cells of Farris are pluripotent would not allow one of ordinary skill in the art to reasonably conclude that the isolated cells are in fact stem cells and that they are hepatic stem cells. Applicants' arguments are respectfully found to be unpersuasive for the following reasons.

Firstly, "stem cell" as defined by Faris as an undifferentiated cell that differentiate into a mature functional hepatocyte or bile duct cells (see col. 1, lines 37-39), and therefore, the composition of isolated liver cell cluster of Faris contain cells having pluripotent and hepatic characteristics.

Secondly, claims of the issued patent No. 6,129,911 are valid until proven otherwise.

Thirdly, Examiner notes that Applicants have also not demonstrated the pluriptotent characteristics for any of the isolated cell populations in the present application.

Accordingly, amended claims 11, 20, 21-26 and 42-44 are rejected for the reasons set forth above.

Claim Rejections - 35 USC § 103

Amended claims 1-6, 8, 12-19 and 45-48 are rejected under 35 U.S.C. 103(a) as being unpatentable over Reid et al. (U.S. Patent No. 6,069,005) in view of Mitaka et al. (Biochem. Biophys. Res. Comm. 214:310-317, 1997; IDS) and Naughton et al. (U.S. Patent No. 5,559,022; IDS).

Reid et al. disclosed a method of isolating hepatic progenitors from rat fetal livers utilizing panning techniques and flow cytometry on single cell suspension of liver cells (See claim 1, column 20). The method comprises the panning and fluorescence activated cell sorting of fetal liver cells using specific antibodies to remove mature hepatocytes, mature bile duct cells, endothelial cells, mesenchymal cells and hemapoietic cells for obtaining a cell population enriched for immature hepatic cell types which were subsequently separated into distinct subcategories by multiparametric fluorescence activated cell sorting (See Examples I and II). The panning stage involves multiple steps (see Table 3 in col. 6, for example) resulting in isolated cells enriched up to 5-fold for AFP mRNA and 2-fold for albumin mRNA (col. 17, lines 19-27). One of the panning steps is a selecting step for cells exhibiting one or more markers indicative of expression of alpha-protein, albumin or both, for this instance mRNAs of AFP and albumin. Since a product and its properties can not be separated, hepatic progenitors isolated by the disclosed method also possess markers indicative of expression of

alpha-fetoprotein, albumin, or both (full-length mRNAs, for examples), as well as alpha-fetoprotein-like immunoreactivity, albumin-like immunoreactivity, or a combination thereof as evidenced by the enrichment of AFP mRNA and albumin mRNA in selected cells after the panning. It is further noted that fetal liver cells selected for flow cytometry in the disclosed method have a broad range in cell size, 5 to 15 microns (See column 17, lines 50-51).

Reid et al. did not specifically teach a method of providing a composition comprising a mixture of cells derived from human liver tissue or an enriched population of human hepatic progenitors. However, Reid et al. state that their method offers a systematic approach to isolating hepatoblasts (hepatic progenitors) from any age from any species (column 2, lines 45-49). Reid et al. also do not disclose a step for debulking the cell suspension based on size, buoyant density, or a combination thereof to remove mature cells, while retaining immature cells in the isolation of hepatic progenitors.

However, at the filing date of the present application, Mitaka et al. teach an isolating method for small hepatic "committed progenitor" cells involving the removal of a pellet of parenchymal cell fraction using low speed centrifugation at 50 x g for 1 min (page 311, see the section on "Isolation of Cells"). Naughton et al. teach the isolation and characterization of a liver progenitor cell population with high proliferative activity and ability to differentiate *in vitro* from human livers (see the entire patent, particularly col. 5, line 66 continues to line 4 of col. 6).

Accordingly, it would have been obvious and within the scope of skill for an ordinary skilled artisan at the time of invention was made to modify the method disclosed by Reid et al. by replacing rat fetal liver tissue as the starting material with human liver tissue, and introducing a low speed centrifugation step in the modified isolation method to remove the bulk of parenchymal cells from smaller-sized hepatoblasts or hepatic progenitors in light of the teachings of Mitaka et al. and Naughton et al.

One of ordinary skilled artisan would have been motivated to carry out the above modifications to obtain a composition enriched in a population of human liver progenitors or human hepatic pluripotent progenitors for cellular characterization as well as for cell transplantation studies. Additionally, one of ordinary skilled artisan would have been motivated to further introduce a low speed centrifugation step in the modified isolation procedure to remove the bulk of large mature hepatocytes or parenchymal cells from progenitor cell populations (5-15 micron in diameter), so that less contaminating large sized parenchymal cells are present in the cell suspension subjected to panning and fluorescence activated cell sorting procedures.

Thus, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Amended claims 21 and 38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Muench et al. (Blood 83:3170-3181, 1994) or Muench et al. (Blood 89:1364-1375, 1997) in view of Reid et al. (U.S. Patent No. 5,789,246, PTO-1449 AB).

The claims are drawn to a composition comprising an enriched population of human hepatic pluripotent progenitors, their progeny, or more mature forms thereof, which exhibit one or more markers indicative of expression of full-length alpha-fetoprotein, full-length albumin, or both; and a cell culture comprising the same composition, an extracellular matrix component, and a culture medium (Claim 38).

Muench et al. (1994) disclose the isolation of human fetal liver progenitors with a high proliferative potential and a phenotype of $CD34^+$, $CD33^+$, $CD13^+$, CD38-, lin^- (lineage= CD3, CD8, CD10, CD14, CD15, CD16, CD19, CD20 and glycophorin A), $CD45RA^-$, $CD45RO^-$, $CD71^-$, and heterogeneous *for c-kit* or CD117 (See abstract and page 3171). Additionally, Muench et al. (1994) disclose the isolation of $CD34^+$, $CD33^-$ HLA-DR-, CD38- cell population previously suggested to contain stem cells (see abstract and page 3179, col. 2). Muench et al. (1997) disclose the isolation of hematopoietic stem cells derived from human fetal liver, with a phenotype of $CD4^+$, $CD34^{++}$, Lin^-, $CD117^+$, $CD38^-$, $CD45RA^-$ (See abstract and page 1365). Since a product and its properties can not be separated, human fetal liver progenitors and human hematopoietic stem cells derived from human fetal liver isolated by Muenche et al. (1994, 1997) also possess the same properties as the enriched population of human hepatic pluripotent progenitor cells of the presently claimed invention. However, Muench et al. (1994, 1997) did not teach a cell culture comprising these cell populations, an extracellular matrix component, and a culture medium.

However, Reid et al. taught a cell culture comprising hepatocyte precursors being plated on or in a matrix of collagen type IV and in the serum-free, hormonally defined medium (See columns 2-4) for the expansion or proliferation of hepatocyte precursors.

Accordingly, it would have been obvious to a person of ordinary skill in the art at the time of invention was made to adopt the cell culture system taught by Reid et al. for the expansion of human fetal liver progenitors and hematopoietic stem cells derived from human fetal liver disclosed by Muench et al. (1994, 1997). An ordinary skilled artisan would have been motivated to carry out the above modification for expanding human fetal liver progenitors and hematopoietic stem cells for uses in artificial livers, for toxicology and pharmacology studies (See abstract in Reid et al.).

Thus, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on September 30, 2002 in Paper No. 16 (page 10) have been fully considered.

Applicants argue basically that the disclosures of Muench et al. fail to teach the separation of mature cells from immature cells and only disclose the alleged isolation of hematopoietic progenitors. Additionally, the claimed enriched population of human hepatic pluripotent progenitors, their progeny, or more mature forms thereof, exhibit one or more markers indicative of expression of full-length alpha-fetoprotein, full length albumin, or both, and in contrast the inventors found that a truncated form of alpha-

fetoprotein, which is missing the N-terminal portion of the protein is expressed by a hematopoietic cell line, and that similar results were also observed for albumin. Applicants' arguments are respectfully found unpersuasive for the following reasons.

Firstly, the human hepatic pluripotent progenitors of the presently claimed invention are indistinguishable over the isolated human fetal liver progenitor cell populations of Muench et al. (1994) and Muench et al. (1997) by sharing the same common cell surface antigens. It is further noted that a product and its properties can not be separated. Furthermore, the human hepatic pluripotent progenitors of the presently claimed invention comprise hemopoietic progenitors

Secondly, the observation that truncated albumin and alpha-fetalprotein RNA messages are found in a specific human erythroleukemia cell line K562 does not necessarily indicate that human hematopoietic progenitor cell populations would also contain truncated albumin and alpha-fetoprotein RNA messages. This is because the erythroleukemia K562 cells are not the same cells as human hematopoietic progenitor cell populations taught by Muench et al. (1994, 1997). Furthermore, there is no factual evidence indicating that hematopoietic progenitors also contain truncated mRNA messages for albumin and alpha-fetoprotein.

Accordingly, amended claims 21 and 38 are rejected for the reasons set forth above.

Double Pat nting

Amended claims 27-33 remain provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 59-77 of copending Application No. 09/154224. Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant claims encompass all the embodiments of the pending claims of the co-pending Application No. 09/154224.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Amended claim 35 remains provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 21-39 of copending Application No. 09/534487. Although the conflicting claims are not identical, they are not patentably distinct from each other because a method of treating a disease in a subject in need thereof using an effective amount of human hepatic progenitors, their progeny or more mature forms thereof in which the human hepatic progenitors, their progeny, or more mature forms harbor exogenous nucleic acid of the instant application encompass the embodiments of a method of treatment of liver dysfunction in a subject in need thereof using a genetically engineered hepatocyte precursor to the subject in the co-pending Application No. 09/534487.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Examiner notes that Applicants will consider the possibility and propriety of filing a terminal disclaimer upon the allowance of the '224 Application or upon the allowance of '487 Application.

Conclusions

No claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (703) 308-8339.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's mentor, David Guzo, Ph.D., may be reached at (703) 308-1906, or SPE, Irem Yucel, Ph.D., at (703) 305-1998.

Any inquiry of a general nature or relating to the status of this application should be directed to LIE, Tiffany Tabb, whose telephone number is (703) 605-1238.

Quang Nguyen, Ph.D.

DAVID GUZO
PRIMARY EXAMINER